

PATENT SPECIFICATION

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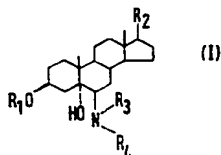


(54) 6 β -AMINO-STEROIDS AND THE PREPARATION THEREOF

(71) We, SHIONOGI & CO LTD, a Japanese Body Corporate, of 12 3-chome, Dosho-machi, Higashi-ku, Osaka, Japan, do hereby declare this invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

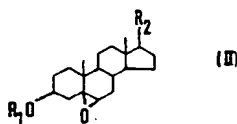
This invention relates to 6 β -amino-steroids and acid addition or quaternary ammonium salts thereof which are useful as hypocholesterolemic agents, hypolipidemic agents, anti-tumor agents or anti-viral agents, and to the preparation thereof.

According to the present invention there is provided a 6 β -amino-steroid of the formula:

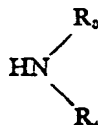


wherein R₁ is hydrogen or acyl; R₂ is hydrogen or an aliphatic hydrocarbyl group; and R₃ and R₄ are each independently hydrogen, adamantyl or alkyl, or, when considered together with the adjacent nitrogen atom, and with or without another hetero atom, form a nitrogen-containing saturated heterocyclic ring.

The invention also provides a process for the preparation of a 6 β -amino-steroid in accordance with the invention or of a salt thereof, which process comprises reacting a 5 α ,6 α -epoxy-steroid of the formula:



wherein R₁ and R₂ are as hereinbefore defined with an amine of the formula:



wherein R₃ and R₄ are as defined in claim 1, followed, if desired, by hydrolysis, when a 3-acyloxy group is present, to produce a 3-hydroxy group, acylation, when a 3-hydroxy group is present, to produce a 3-acyloxy group, or formation of a salt of the resultant product in a manner known *per se*.

In the formulac, R₁ may be hydrogen or acyl e.g. an alkanoyl group (e.g. formyl,

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acetyl, or propionyl), an aralkanoyl group (e.g. phenylacetyl or phenylpropionyl), or an aryloyl group (e.g. benzoyl, toluoyl, *o*-carboxy-benzoyl). The substituent represented by OR₁ has the α - or β -configuration. R₂ is a hydrogen atom or an aliphatic hydrocarbonyl group such as an alkyl or alkenyl group, having preferably up to 10 carbon atoms. Preferred hydrocarbonyl groups are methyl, ethyl, and the hydrocarbon substituents at position 17 of cholesterol, β -sitosterol or stigmasterol. R₃ and R₄ each represent adamantyl, alkyl or hydrogen, and preferred alkyl groups are those having up to 10 carbon atoms such as methyl, ethyl, propyl, isopropyl, butyl, pentyl or hexyl. R₃ and R₄ may alternatively form a nitrogen-containing heterocyclic ring such as a piperidino, pyrrolidino, piperazino, morpholino, thiomorpholino, or N-alkyl-piperazino group when taken together with the adjacent nitrogen atom, with or without another hetero atom.

The process of this invention can be performed by reacting a 5 α ,6 α -epoxy-steroid (II) and an amine (III) in the presence or absence of a solvent. The reaction is generally conducted with heating at a temperature of from 50°C to 300°C, preferably 100°C to 200°C. The reaction is usually carried out in a polar solvent having a relatively high boiling point. Examples of such solvents are ethylene glycol, propylene glycol, dimethylformamide, dimethylsulfoxide, alcohols with high boiling points (e.g. butyl alcohol, tertiary alcohol, or amyl alcohol) and water. However, the reaction can proceed without such solvents, and an amount of amine (III) in excess of that undergoing reaction may serve as the reaction solvent. The present reaction is complete within a few to several tens of hours, depending on the starting materials, solvent, and reaction temperature used. If necessary, the reaction may be carried out in an atmosphere of inert gas such as nitrogen or helium. After completion of the reaction, the desired 6 β -amino-steroid (I) may be separated and purified by solvent extraction, evaporation under reduced pressure, recrystallization, chromatography, or by any other conventional method, or by a combination of such methods.

The starting material, the 5 α ,6 α -epoxy-steroid (II), can be prepared by epoxidizing a corresponding Δ^5 -steroid with an organic peracid according to the method described in *Helv. Chim. Acta* 20, 244 (1937), *Ann. Chem.* 508, 215 (1934) or *J. Chem. Soc.* p. 738 (1936).

Examples of the other starting material in the present process, the amine (III), are primary, secondary, and tertiary amines such as ammonia, adamantylamine, alkylamines (e.g. methylamine, ethylamine, propylamine, isopropylamine, butylamine, tert-butylamine, dimethylamine, diethylamine, dipropylamine, dibutylamine or methylisopropylamine), and heterocyclic amines (e.g. piperidine, piperazine, pyrrolidine, morpholine, thiomorpholine, or N-alkyl-piperazines in which the alkyl moiety contains up to 6 carbon atoms).

When R₁ in formula (I) stands for a hydrogen atom and/or at least one of R₃ and R₄ is a hydrogen atom, the amino steroid (I) may be 3-*o*-acylated, if desired. This acylation may be performed according to usual methods, and is usually carried out in pyridine or other suitable basic solvent, although it may also proceed in a neutral solvent. The acylation may be accelerated by the addition of a suitable inorganic or organic basic substance. Examples of acylating agents which may be used are acid anhydrides or acid halides of alkanic acids (e.g. formic acid, acetic acid, or propionic acid), aralkanonic acids (e.g. phenylacetic acid or phenylpropionic acid), or arylcarboxylic acids (e.g. benzoic acid or phthalic acid). Depending on the reaction conditions, starting materials, and acylating agents used, the acylation takes place at the hydroxy group of position 3 and/or at the amino group of position 6 β in the steroid nucleus. The acylated product can be separated and isolated by conventional methods involving, for example, recrystallization, fractional precipitation, or chromatography. In general, when the acylation is effected with acetic anhydride under mild conditions in pyridine at about room temperature, the hydroxy group at position 3 is predominantly acylated. In addition, the amino-steroid (I) in which R₁ is an acyl group may be optionally deacylated by any known method to give the corresponding 3-hydroxy compound (I, R₁ = H).

The thus-obtained 6 β -amino-steroids (I) can be, after or without isolation, converted into inorganic or organic acid addition salts which are valuable for pharmaceutical purposes. Among suitable salts are the hydrochlorides, hydroiodides, sulfates, nitrates, phosphates, carbonates, formates, acetates, propionates, oxalates, succinates, tartrates, malates, citrates, benzoates, and salicylates of the 6 β -amino-steroids (N). Also, the amino-steroids of the invention may be converted into quaternary ammonium salts by known methods using alkyl halides.

The 6 β -amino-steroids of the invention possess strong hypolipidemic and hypocholesterolemic activities and they cause marked decreases in plasma levels of cholesterol, phospholipids, and triglycerides, and in the cholesterol/phospholipid ratio. For

example, the test results on 6 β -isopropylamino-5 α -cholestane-3 β ,5-diol (A) and 6 β -isopropylamino-stigmast-22-en-3 β ,5 α -diol (B) are summarized in the following table:—

TABLE

Group	Control	A	B
No. of rats	7	7	7
Body weight (g \pm S.E.)			
Initial	266 \pm 6.0	265 \pm 3.8	266 \pm 3.8
Final	295 \pm 8.1	286 \pm 5.0	289 \pm 4.7
Gain (mean)	29 \pm 3.0	20 \pm 2.4	23 \pm 2.5
Plasma:			
Cholesterol (mg/dl \pm S.E.)	57.9 \pm 0.9	42.3 \pm 0.7 (26.9%)	48.1 \pm 1.3 (16.9%)
Phospholipid (mg Eq./dl \pm S.E.)	106.8 \pm 4.6	86.4 \pm 1.9 (19.1%)	102.0 \pm 1.8 (4.5%)
Triglyceride (mg/dl \pm S.E.)	30.5 \pm 2.2	17.8 \pm 1.2 (41.6%)	28.4 \pm 2.9 (6.9%)
Cholesterol/phospholipid (mean \pm S.E.)	0.53 \pm 0.01	0.49 \pm 0.01 (7.5%)	0.49 \pm 0.01 (7.5%)
Liver:			
Weight (g/100 g body weight)	3.93 \pm 0.11	3.70 \pm 0.07	4.25 \pm 0.05
Cholesterol	2.70 \pm 0.06	2.72 \pm 0.05	2.76 \pm 0.06
Phospholipid	30.0 \pm 0.6	31.0 \pm 0.6	30.9 \pm 0.6
Diet uptake (g./day/rat)	18.6	16.5	16.9

Note: The numbers in parentheses of the "plasma" columns are percent decreases. The test results were obtained in the following manner: Wistar male rats weighing 250–280 g were fed a diet with or without 0.03% test compounds daily for two weeks, and the decreases of lipid levels in plasma and liver were studied by colorimetric determination.

As exemplified in the above table, the 6 β -amino-steroids of the invention cause marked decreases in the levels of plasma cholesterol and lipids. Also, such steroids do not show any significant effect on liver weight, liver cholesterol and liver phospholipid. Clofibrate, a well known hypolipidemic agent, has undesirable effects on liver. Thus, these properties of the present 6 β -amino-steroids are valuable in clinical applications.

Another advantage of the present 6 β -amino-steroids is that they do not inhibit normal cholesterol biosynthesis. Thus, gas chromatographic analysis revealed no accumulation of cholesterol biosynthesis intermediates such as desmosterol, which can usually be detected in the metabolites of nitrogen-containing steroids, in rats which had

been subcutaneously injected with 6 β -isopropylamino-5 α -cholestane-3 β ,5-diol in a dose of one mg/day/rat for 10 days.

The acute toxicity of the present 6 β -amino-steroids was studied using DS mice weighing 20—23 g. The drugs were administered by subcutaneous or oral routes in various doses of the test compounds and the mice were placed in a cage. The mortality was determined 72 hours after administration. The acute toxicities (LD₅₀ value) of 6 β -isopropylamino-5 α -cholestane-3 β ,5-diol and 6 β -isopropylamino-stigmast-22-en-3 β ,5 α -diol were both higher than 1000 mg/kg.

It should be noted that the test data described above have been given for certain steroids only. Since the other compounds of this invention as well as those used in the tests described above have similar characteristics and advantages as medicaments, the 6 β -amino-steroids of the present invention are highly useful and advantageous in the treatment or prevention of hypercholesterolemia, hyperlipidemia, atheromatous conditions and atherosclerosis in human beings and domestic animals.

The present 6 β -amino-steroids can be administered orally or parenterally in conventional dosage forms, e.g. tablets, granules, powders, injections, liquids, suspensions or emulsions. They are optionally administered with suitable carriers, stabilizers, emulsifiers, preservatives, buffers, isotonicizing agents and/or wetting agents in compositions containing therapeutically active amounts of the active ingredient.

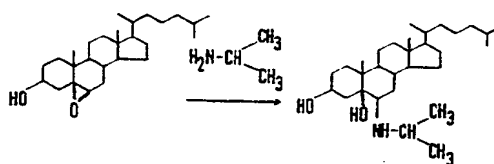
Thus, the invention also provides a pharmaceutical composition comprising a 6 β -amino-steroid or a salt thereof in accordance with the invention together with a pharmaceutically acceptable diluent or carrier.

Furthermore, the invention includes a method for reducing plasma lipid levels or plasma cholesterol levels in an animal, which method comprises administering to the animal an effective dose of a 6 β -amino-steroid or a salt thereof in accordance with the invention, or of a pharmaceutical composition in accordance with the invention.

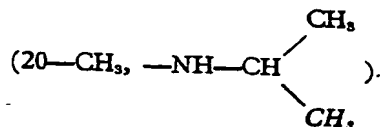
The effective dose can be easily determined by a physician on the basis of the data herein described. Thus, a typical clinical dosage range of the present 6 β -amino-steroids is approximately several hundred γ to several grams, preferably 300 mg to 1000 mg, for a normal adult.

The invention will now be further described and illustrated by way of specific Examples. Example 13 is not illustrative of the invention but is given by way of reference.

EXAMPLE 1.



A suspension of 5 α ,6 α -epoxy-cholesterol (6.5 g), triethylene glycol (160 ml) and isopropylamine (26 ml) is heated under an atmosphere of nitrogen gas at 180°C for 15 hours. The reaction solution is added to ice-water then extracted with chloroform. The chloroform extract is washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a residue. The residue is recrystallized from ethanol to give 4.54 g of 6 β -isopropylamino-5 α -cholestane-3 β ,5-diol, m.p. 170—171°C, $[\alpha]_D^{25} -15.5^\circ$ (c 0.5, chloroform), IR (KBr): 3528 (OH), 3282 (OH), 3192 (NH) cm⁻¹, NMR (CDCl₃) δ : 0.64 (18—CH₃), 0.73 (19—CH₃), 0.88, 0.86 (26—CH₃, 27—CH₃), 0.92, 0.96, 0.98, 1.02

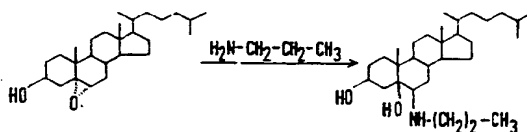


Anal. Calcd. for C₃₀H₅₂O₂N: C, 78.0; H, 12.0; N, 3.0.
Found: C, 78.2; H, 12.1; N, 3.2.

The product (50 mg) obtained above is dissolved in chloroform saturated with dry hydrogen chloride and the solution is evaporated under reduced pressure to give crystals. Recrystallization from methanol-ethyl acetate gives 6 β -isopropylamino-5 α -cholestane-3 β ,5-diol hydrochloride in quantitative yield, m.p. 196—197°C.

To a solution of 6 β -isopropylamino-5 α -cholestane-3 β ,5 α -diol (100 mg) in methylene chloride (2 ml) is added 20% acetic acid (10 ml), and the mixture is stirred. After removal of the methylene chloride layer, an aqueous saturated solution of sodium chloride is added to the aqueous layer to precipitate crystals, which are recrystallized from methanol-ether to give the corresponding acetate, m.p. 193—194°C.

EXAMPLE 2.

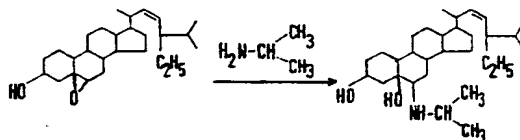


In the same manner as in EXAMPLE 1, but using 5 α ,6 α -epoxy-cholesterol (0.3 g), triethylene glycol (5 ml) and propylamine (5.0 ml), 0.145 g of 6 β -propylamino-5 α -cholestane-3 β ,5 α -diol is obtained, m.p. 149—152°C, $[\alpha]_D^{25} - 14.1^\circ$ (c 0.3, chloroform).

The product obtained is dissolved in dry chloroform, then chloroform saturated with hydrogen bromide is added. The mixture is allowed to stand at room temperature then evaporated under reduced pressure. The resultant residue is recrystallized from an aqueous ethanol to give 6 β -propylamino-5 α -cholestane-3 β ,5 α -diol hydrobromide in quantitative yield, m.p. 218—220°C (decomp.).

Anal. Calcd. for $C_{30}H_{57}O_2N \cdot HBr \cdot H_2O$: C, 64.2; H, 10.4; N, 2.4.
Found: C, 64.4; H, 10.4; N, 2.5.

EXAMPLE 3.

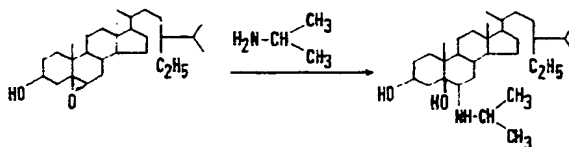


5 α ,6 α -Epoxy-stigmasterol (6.5 g) is dissolved in triethylene glycol (220 ml), and isopropylamine (23 ml) is added. The mixture is heated at 175—180°C for 18 hours. When the reaction is complete, the mixture is treated as in EXAMPLE 1 to give a residue. The residue is recrystallized from methylene chloride-n-hexane to afford 6 β -isopropylamino-stigmaster-22-en-3 β ,5 α -diol, yield 4.0 g, m.p. 178—179°C, $[\alpha]_D^{24} - 24.2^\circ$ (c 0.5, chloroform).

Anal. Calcd. for $C_{32}H_{57}O_2N$: C, 78.8; H, 11.8; N, 2.8.
Found: C, 78.9; H, 11.8; N, 2.9.

The product (50 mg) obtained above is dissolved in methylene chloride (10 ml) then 10% tartaric acid is added with stirring. The aqueous phase is separated then to it is added dropwise a saturated aqueous sodium chloride solution. The resultant crystals are collected by filtration and recrystallized from methanol-ether to give 6 β -isopropylamino-stigmaster-22-en-3 β ,5 α -diol tartrate in quantitative yield, m.p. 194—196°C.

EXAMPLE 4.



On using 5 α ,6 α -epoxy- β -sitosterol (0.3 g), triethylene glycol (6 ml), and isopropylamine (0.8 ml), the same reaction as described in EXAMPLE 1 gives 6 β -iso-

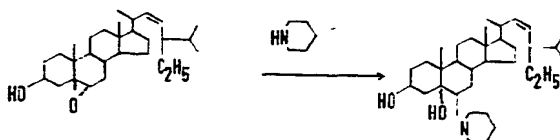
propylamino-stigmastane-3 β ,5 α -diol, m.p. 156°—157°C, $[\alpha]_D^{24}$ -12.1° (c 0.5, chloroform):

Anal. Calcd. for $C_{32}H_{50}O_2N$: C, 77.5; H, 12.1; N, 2.9.
Found: C, 78.1; H, 12.1; N, 2.7.

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EXAMPLE 5.

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Triethylene glycol (5 ml) and piperidine (0.3 ml) are added to 5 α ,6 α -epoxy-stigmasterol (0.3 g) and the mixture is heated at 165—170°C for 7 hours. After completion of the reaction, the mixture is poured into ice-water then extracted with chloroform. The extract is submitted to thin-layer chromatography on silica gel in a solvent system of chloroform:ethyl acetate:n-hexane=2:1:0.5 (v/v), thus yielding 0.11 g of 6 β -piperidino-stigmast-22-en-3 β ,5 α -diol, m.p. 167—169°C, $[\alpha]_D^{24}$ -20.5° (c 0.6, chloroform), IR (chloroform): 3350—3500 cm^{-1} .

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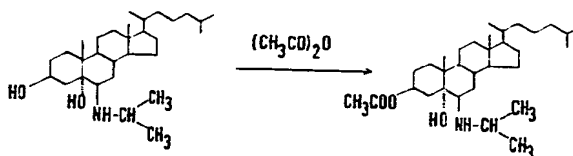
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Anal. Calcd. for $C_{34}H_{50}O_2N$: C, 79.4; H, 11.5; N, 2.7.
Found: C, 79.2; H, 11.4; N, 2.7.

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EXAMPLE 6.



6 β -Isopropylamino-5 α -cholestane-3 β ,5 α -diol (50 mg) obtained as in EXAMPLE 1 is added to a mixture of pyridine (1 ml) and acetic anhydride (0.5 ml) and the mixture is allowed to stand at room temperature. The product obtained is recrystallized from ether to give 35.4 mg of 3 β -acetoxy-6 β -isopropylamino-cholestan-5 α -ol, m.p. 76—77°C, $[\alpha]_D^{22}$ -20.5° (c 0.5, chloroform), IR (chloroform): 3300—3500, 1735 cm^{-1} .

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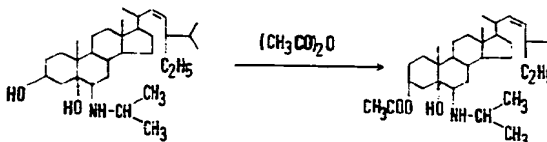
20

Anal. Calcd. for $C_{32}H_{50}O_3N$: C, 76.2; H, 11.3; N, 2.7.
Found: C, 76.1; H, 11.1; N, 2.9.

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EXAMPLE 7.



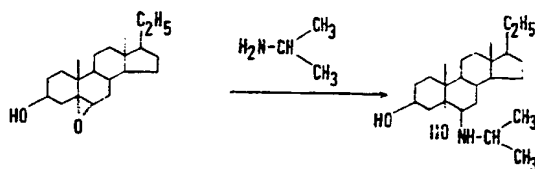
6 β -Isopropylamino-stigmast-22-en-3 β ,5 α -diol (50 mg), obtained as in EXAMPLE 3, is acetylated in the same manner as in EXAMPLE 6 to produce 3 β -acetoxy-6 β -isopropylamino-stigmast-22-en-5 α -ol, yield 34.8 mg, m.p. 81—82°C, $[\alpha]_D^{23}$ -25.6° (c 0.4, chloroform), IR (chloroform): 3300—3500, 1735, 970 cm^{-1} .

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Anal. Calcd. for $C_{34}H_{50}O_3N$: C, 77.0; H, 11.2; N, 2.6.
Found: C, 76.9; H, 11.2; N, 2.5.

EXAMPLE 8.

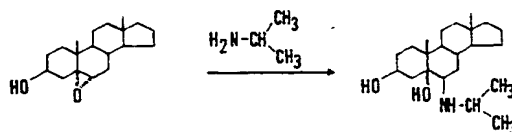


5,6α-Epoxy-5α-pregnan-3β-ol (5.6 g) is added to triethylene glycol (450 ml) containing isopropylamine (20 ml) and the mixture is heated in an oil bath at about 190°C for 18 hours. Working up in the same manner as in EXAMPLE 1, 3.4 g of 6β-isopropylamino-5α-pregnane-3β,5-diol is obtained, m.p. 195—196°C (recryst. from methanol), $[\alpha]_D^{23.5} - 33.3^\circ$ (c 0.5, chloroform).

Anal. Calcd. for $C_{24}H_{40}O_2N$: C, 76.3; H, 11.4; N, 3.7.
Found: C, 76.5; H, 11.4; N, 3.7.

The starting material, 5,6α-epoxy-5α-pregnan-3β-ol can be prepared by the following procedure. To a solution of 5-pregnen-3β-ol (6.8 g) in methylene chloride (150 ml) is added dropwise a solution of *m*-chloroperbenzoic acid (4.5 g) in methylene chloride (150 ml) with stirring at 0°C. After 3 hours, the reaction solution is treated in a conventional manner and the residue is crystallized from methanol, thus yielding 5,6α-epoxy-5α-pregnan-3β-ol, yield 5.7 g, m.p. 147—148°C, $[\alpha]_D^{23.5} - 59.0^\circ$ (c 0.49, chloroform).

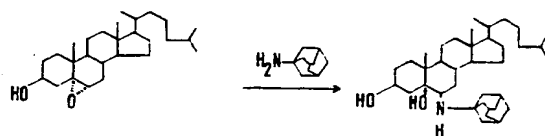
EXAMPLE 9.



5,6α-Epoxy-5α-androstan-3β-ol (4.0 g) prepared by the method described in Ber. 75, 597 (1942) is heated in triethylene glycol (200 ml) together with isopropylamine (4.0 ml) at 175—180°C for 10 hours. The reaction mixture is then poured into ice-water and extracted with chloroform. After removal of the solvent, the residue is recrystallized from methylene chloride-*n*-hexane to give 6β-isopropylamino-5α-androstane-3β,5-diol, yield 1.941 g, $[\alpha]_D^{21} - 47.9^\circ$ (c 0.4, chloroform).

Anal. Calcd. for $C_{22}H_{36}O_2N$: C, 75.6; H, 11.2; N, 4.0.
Found: C, 75.7; H, 11.2; N, 4.1.

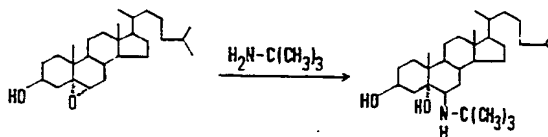
EXAMPLE 10.



5α,6α-Epoxy-cholestan-3β-ol (200 mg) is heated together with adamantylamine (150 mg) and water (1.2 ml) in a sealed tube at 140—145°C for 15 hours. The reaction solution is extracted with a mixed solvent of methylene chloride-methanol. The organic extract is dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a residue. The residue is recrystallized from methylene chloride-methanol to give 6β-adamantylamino-5α-cholestane-3β,5-diol, yield 92 mg, $[\alpha]_D^{24.5} - 7.9^\circ$ (c 0.5, chloroform).

Anal. Calcd. for $C_{37}H_{60}O_2N$: C, 80.2; H, 11.4; N, 2.5.
Found: C, 79.9; H, 11.4; N, 2.5.

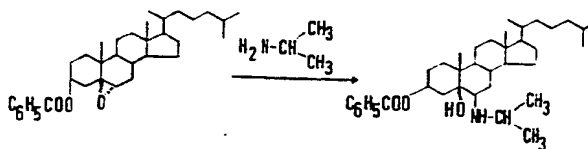
EXAMPLE 11.



5 $5\alpha,6\alpha$ -Epoxy-cholestan-3 β -ol (0.5 g) is added to triethylene glycol (20 ml) then
tert-butylamine (8 ml) is added. The mixture is heated in an oil bath at about 180—
185°C for 20 hours. Work-up in the same manner as in EXAMPLE 1 gives 0.28 g of
6 β -tert-butylamino-5 α -cholestane-3 β ,5-diol, m.p. 166—167°C (recryst. from ethanol),
5 $[\alpha]_D^{24} - 9.9^\circ$ (c 0.4, chloroform).

Anal. Calcd. for $C_{31}H_{53}O_2N$: C, 78.2; H, 12.0; N, 2.9.
Found: C, 78.0; H, 11.9; N, 2.7.

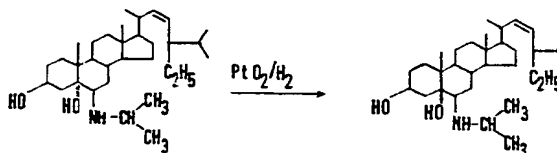
EXAMPLE 12.



15 $5,6\alpha$ -Epoxy-5 α -cholestan-3 β -ol benzoate (0.5 g) is dissolved in triethylene glycol
(5 ml) and to this solution is added isopropylamine (0.5 ml). The mixture is heated at
180°C for about 18 hours. Work-up in the same manner as in EXAMPLE 1 gives
0.34 g of 3 β -benzoyloxy-6 β -isopropylamino-5 α -cholestan-5-ol, m.p. 174—176°C (re-
cryst. from methanol), $[\alpha]_D^{25} - 2.4^\circ$ (c 0.47, chloroform); IR (chloroform): 3300—
3500, 1780, 1610, 1590 cm^{-1} .

Anal. Calcd. for $C_{37}H_{55}O_3N$: C, 78.5; H, 10.5; N, 2.4.
Found: C, 78.4; H, 10.3; N, 2.4.

20 The product obtained above can also be prepared similarly as described for EX-
AMPLE 6 by treating 6 β -isopropylamino-5 α -cholestane-3 β ,5-diol with benzoyl chlor-
ide, in place of the acetic anhydride used in EXAMPLE 6.

EXAMPLE 13.
(EXAMPLE FOR REFERENCE)

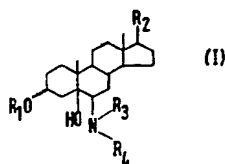
25 6 β -Isopropylamino-stigmast-22-en-3 β ,5 α -diol (0.2 g) is added to a mixture of
ether and acetic acid (1:3 (v/v), 20 ml). After addition of platinum oxide (50 mg), the
mixture is shaken in a hydrogen stream at normal pressure. After three hours, the cata-
lyst is filtered off and the filtrate is adjusted to weakly alkaline pH with a dilute sodium
30 carbonate solution. The alkaline solution is extracted with methylene chloride. The or-
ganic extract is treated in a conventional manner, to yield 6 β -isopropylamino-stig-
mastane-3 β ,5 α -diol, m.p. 155—156°C (recryst. from methylene chloride-n-hexane).
The product obtained is found to be identical to the compound obtained in EXAMPLE
4 by comparison of infrared absorption spectrum and mixed melting point.

35 In so far as the present invention includes within its scope a method for reducing
plasma lipid levels or plasma cholesterol levels in an animal, which method comprises
administering to the animal an effective dose of a 6 β -amino-steroid or a salt thereof in

accordance with the invention, or of a pharmaceutical composition in accordance with the invention, it should be understood that we make no claim herein to such a method when used in the treatment or prevention of disease in human beings.

Subject to the foregoing disclaimer, WHAT WE CLAIM IS:—

1. A 6 β -amino-steroid of the formula:



wherein R₁ is hydrogen or acyl; R₂ is hydrogen or an aliphatic hydrocarbyl group; and R₃ and R₄ are each independently hydrogen, adamantyl or alkyl, or, when considered together with the adjacent nitrogen atom, and with or without another hetero atom, form a nitrogen-containing saturated heterocyclic ring.

2. A 6 β -amino-steroid as claimed in claim 1, wherein R₁ is hydrogen, alkanoyl, aryloyl or aralkanoyl; R₂ is hydrogen or an aliphatic hydrocarbyl group having up to 10 carbon atoms; and R₃ and R₄ are each independently hydrogen or alkyl having up to 10 carbon atoms, or, when considered together with the adjacent nitrogen atom, and with or without another hetero atom, form a piperidino, piperazino, pyrrolidino, morpholino, thiomorpholino, or N-alkylpiperazino group.

3. A 6 β -amino-steroid as claimed in claim 2, wherein the group R₁O has the β -configuration.

4. A 6 β -amino-steroid as claimed in claim 3, wherein R₁ is hydrogen.

5. A 6 β -amino-steroid as claimed in any one of claims 1 to 3, wherein R₁ is formyl, acetyl, propionyl, phenylacetyl, phenylpropionyl, benzoyl, toluoyl, or *o*-carboxy-benzoyl.

6. A 6 β -amino-steroid as claimed in any one of the preceding claims, wherein R₂ is an alkyl or alkenyl group.

7. A 6 β -amino-steroid as claimed in any one of the preceding claims, wherein R₃ and/or R₄ are methyl, ethyl, propyl, isopropyl, butyl, pentyl, hexyl, or adamantyl.

8. 6 β -Propylamino-5 α -cholestane-3 β ,5-diol.

9. 6 β -Isopropylamino-5 α -cholestane-3 β ,5-diol.

10. 6 β -Isopropylamino-stigmast-22-en-3 β ,5 α -diol.

11. 6 β -Isopropylamino-stigmastane-3 β ,5 α -diol.

12. 6 β -Piperidino-stigmast-22-en-3 β ,5 α -diol.

13. 6 β -Isopropylamino-5 α -pregnan-3 β ,5-diol.

14. 6 β -Isopropylamino-5 α -androstane-3 β ,5-diol.

15. 6 β -Adamantylamino-5 α -cholestane-3 β ,5-diol.

16. 6 β -Tert.-butylamino-5 α -cholestane-3 β ,5-diol.

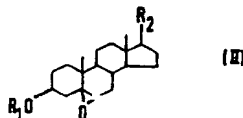
17. 3 β -Acetoxy-6 β -isopropylamino-cholestan-5 α -ol.

18. 3 β -Acetoxy-6 β -isopropylamino-stigmast-22-en-5 α -ol.

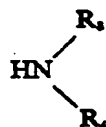
19. 3 β -Benzoyloxy-6 β -isopropylamino-5 α -cholestan-5-ol.

20. An acid addition or quaternary ammonium salt of a 6 β -amino-steroid as claimed in any one of the preceding claims.

21. A process for the preparation of a 6 β -amino-steroid as claimed in claim 1 or of a salt thereof, which process comprises reacting a 5 α ,6 α -epoxy-steroid of the formula:



wherein R₁ and R₂ are as defined in claim 1, with an amine of the formula:



wherein R_3 and R_4 are as defined in claim 1, followed, if desired, by hydrolysis, when a 3-acyloxy group is present, to produce a 3-hydroxy group, acylation, when a 3-hydroxy group is present, to produce a 3-acyloxy group, or formation of a salt of the resultant product in a manner known *per se*.

- 5 22. A process as claimed in claim 21, wherein the reaction between 5 α ,6 α -epoxy-steroid and amine is conducted at a temperature of from 50°C to 300°C. 5
23. A process as claimed in claim 21 or claim 22, wherein the reaction is carried out in a polar solvent having a relatively high boiling point.
- 10 24. A process as claimed in any one of claims 21 to 23, wherein the reaction is carried out in an atmosphere of inert gas. 10
25. A process as claimed in any one of claims 21 to 24, wherein the amine is selected from methylamine, ethylamine, propylamine, isopropylamine, butylamine, tert-butylamine, dimethylamine, diethylamine, dipropylamine, dibutylamine, methyl-isopropylamine, adamantylamine, piperidine, piperazine, pyrrolidine, morpholine, thiomorpholine, or an N-alkyl-piperazine in which the alkyl moiety contains up to 6 carbon atoms. 15
26. A process as claimed in any one of claims 21 to 25, wherein the steroid resulting from the reaction between the 5 α ,6 α -epoxy-steroid and amine has R_1 as hydrogen and this steroid is 3-O-acylated.
- 20 27. A process as claimed in claim 26, wherein the acylation is carried out in the presence of a basic solvent. 20
28. A process as claimed in claim 26 or claim 27, wherein an inorganic or organic base is present to accelerate the acylation.
- 25 29. A process as claimed in any one of claims 26 to 28, wherein the acylating agent used to effect the acylation is selected from acid anhydrides and acid halides of alkanolic acids, aralkanoic acids and arylcarboxylic acids. 25
30. A process as claimed in claim 21 when used to prepare a 6 β -amino-steroid as claimed in any one of claims 2 to 19 or a salt thereof.
- 30 31. A process as claimed in claim 21 and substantially as hereinbefore described in any one of Examples 1 to 12. 30
32. A 6 β -amino-steroid as claimed in claim 1 or a salt thereof which has been prepared in a process as claimed in any one of claims 21 to 29 or 31.
33. A 6 β -amino-steroid as claimed in any one of claims 2 to 19 or a salt thereof which has been prepared in a process as claimed in claim 30.
- 35 34. A pharmaceutical composition comprising a 6 β -amino-steroid or a salt thereof as claimed in any one of claims 1 to 20, 32, or 33, together with a pharmaceutically acceptable diluent or carrier. 35
35. A pharmaceutical composition as claimed in claim 34, also comprising a stabilizer, emulsifier, preservative, buffer, isotonicizing agent and/or wetting agent.
- 40 36. A method for reducing plasma lipid levels or plasma cholesterol levels in an animal, which method comprises administering to the animal an effective dose of a 6 β -amino-steroid or a salt thereof as claimed in any one of claims 1 to 30, 32 or 33, or of a pharmaceutical composition as claimed in claim 34 or claim 35. 40

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